Relationships between plasma amino acid concentrations and blood pressure in South Africans of African descent

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INTRODUCTION

Despite considerable advances in treatment, cardiovascular disease remains a leading cause of death worldwide[1] consequently; improved methods of managing cardiovascular risk are required. An important change associated with vascular pathology is abnormalities of endothelial function and in this regard, changes in the capacity of endothelial cells to synthesise and release nitric oxide (NO) is a key alteration. Twenty years ago NO was identified as an arterial vasodilator[2,3] formed from the semi-essential amino acid, L-arginine, in endothelial cells.[4] Although NO production depends on extracellular arginine,[5] the importance of arginine in cardiovascular diseases is derived mainly from studies showing beneficial effects of supplementation. Indeed, arginine supplementation decreases blood pressure (BP);[6] improves outcomes in patients with heart failure;[7] increases coronary small vessel endothelial function;[8] inhibits atherogenesis;[9,10] and improves hemodynamic responses in hypercholesterolemic men.[11] How-

ABSTRACT

Oral supplementation with the amino acid arginine, the precursor of the vasodilator nitric oxide (NO), is associated with a reduction in blood pressure (BP). However, it is uncertain whether a decreased plasma arginine concentration predicts increases in BP. We assessed the relationship between fasting plasma arginine or other amino acid concentrations and 24 hour ambulatory BP in 75 never-treated participants recruited from the Johannesburg area, 55 of whom were male. Plasma amino acid concentrations were measured with high performance liquid chromatography-mass-spectrometry. Plasma arginine concentrations were not inversely correlated with ambulatory BP. However, plasma arginine concentrations were increased in 36 participants with a mean daytime systolic BP >140 mm Hg (61 ± 17 μmol/L) as compared to the remaining participants (54 ± 15 μmol/L, p<0.05). Moreover, plasma arginine concentrations were positively correlated with 24-hour diastolic BP (r=0.26, p<0.05). In males with a BMI<30kg/m², plasma arginine concentrations were positively correlated with both night diastolic (r=0.46, p<0.005) and systolic (r=0.42, p<0.005) BP. In a multivariate model with adjustments for age gender, body mass index, and other amino acid concentrations, plasma arginine concentrations were independently and positively associated with night diastolic BP (p<0.05). In conclusion plasma arginine concentrations are positively associated with ambulatory BP in a group of participants of African descent in South Africa. These data do not support the notion that deficiencies of arginine, the amino acid substrate for NO, are related to increases in BP in groups of African ancestry living in South Africa. However, as with other ethnic groups, the positive relationship between plasma arginine concentrations and BP suggests a reduced capacity to utilise the amino acid substrate for NO synthesis. SAHeart 2009; 6:142-147

ever, despite data to suggest that increasing the availability of arginine may reduce cardiovascular risk, there is little evidence to indicate that the availability of arginine as a substrate for NO is in fact abnormal.

Although it is thought that circulating arginine concentrations remain constant, low arginine concentrations do occur[12] and, in infectious diseases, where the inducible form of nitric oxide synthase (iNOS) would be active, these decreased concentrations are associated with adverse outcomes in diseases such as cerebral malaria.[13] With respect to cardiovascular diseases, some studies have assessed the relationship between plasma arginine concentrations and BP with outcomes that suggest that, although the availability of arginine is not reduced in hypertension, the capacity to produce NO from arginine may be impaired.[14-16] None of these studies were conducted in groups of African ancestry whom have a higher prevalence of hypertension than their Caucasian counterparts.[17] Consequently, the aim of the present study was to evaluate the relationship between plasma arginine or other amino acids and BP in a group of African descent living in Africa.

METHODS

Study group and BP measurement

A convenience sample of apparently healthy adult South Africans of African ancestry (black South Africans) not receiving antihypertensive or other therapy, and without cardiovascular disease were recruited from outpatient clinics at the Chris Hani-Baragwanath Hospital (participants were referred for screening of cardiovascular disease); inner city clinics (also referred for screening of cardiovascular disease); and from laboratory staff at the National Health Laboratory Services at the Charlotte Maxeke (Johannesburg) Hospital. Participants provided written, informed consent. Ambulatory 24 hour BP measurements were obtained using oscillometric monitors (SpaceLabs, model 90207), programmed to measure BP at 20-minute intervals from 06:00 to 18:00h (daytime) and at 30-minute intervals from 18:00 to 06:00h (night time). An increased day ambulatory BP was defined as ≥ 140 or 90 mm Hg. Routine blood chemistry was performed by the National Health Laboratory Services.

Plasma amino acid measurements

After having fasted from 22:00 hours the previous night, early morning blood samples were obtained from participants and plasma was stored at -70°C. Concentrations of amino acids were determined using high performance liquid chromatography (HPLC)-mass spectrometry (MS) according to previously described techniques.[18,19] Quantification of the amino acids was performed using an Agelient HPLC system using a Phenomenex Prodigy C18 column (4.6x100mm; 3µm particle size) equilibrated with 25mM heptafluorobutyric acid in water containing 5% acetonitrile and 0.1% formic acid (Mobile phase A) at 45°C. Separation was achieved with a gradient of 25mM heptafluorobutyric acid in acetonitrile containing 5% water and 0.1% formic acid. The gradient was as follows: 0-3 minutes: 100% A; 3-9.5 minutes: 30% B; 9.5-13.5 minutes: 32% B; 13.5-14 minutes 60% B with a 4 minutes re-equilibration step. A flow rate of 700µl/min was used and a 10µl injection volume. Eluting peaks were identified by retention time and multiple reactions monitoring using a MSD Sciex 4000QTrap mass spectrometer in positive mode at 5200V.

Data analysis

Analysis was performed using SAS version 9.1 software. Results are reported as mean±SD or as frequencies. Either a Student's t-test or a Kruskal-Wallis test was used to determine differences between groups for normally and non-normally distributed data respectively. Pearson's correlation coefficients were used to determine the association between variables and multivariate regression analysis to assess the independent relationships between plasma arginine concentrations and BP. Primary analysis was conducted in the whole group, but as the majority of participants were male, secondary analysis was performed in males only. As obesity may represent a nutritional abnormality that may alter plasma amino acid profiles, secondary analysis was also conducted in participants with a BMI<30 kg/m^2. A p-value of less than 0.05 was considered to be of statistical significance.

RESULTS

Participant characteristics

Table 1 shows the characteristics of the participants and the differences in the characteristics of participants with a normal and increased day BP. The majority of participants were male (80%). The mean age of the participants was 49 and approximately one third had a history of regular smoking or use of alcohol.

TABLE 1: Demographics of all study participants and comparison of demographic and clinical data between participants with normal and elevated ambulatory blood pressures (ABP).

	All participants (n=75)	Mean Day ABP DBP<90mm Hg (n=33)	Mean Day ABP DBP≥90mm Hg (n=42)	Mean Day ABP SBP<140mm Hg (n=39)	Mean Day ABP SBP≥140mm Hg (n=36)
Male/female	60/15	24/9	36/6	24/9	36/6
Age (years)	49 ± 7	50 ± 7	48 ± 6	50 ± 7	48 ± 7
BMI (kg/m^2)	27.8 ± 4.9	26.8 ± 4.7	28.5 ± 5.0	26.6 ± 4.9	28.9 ± 4.7
Smoking (n (ex-n))	24 (8)	11 (3)	13 (5)	11 (3)	13 (5)
Alcohol (n)	28	12	16	12	16
Mean Ambulatory BP					
24H SBP/ (mm Hg)	135 ± 18/	121 ± 13/	146 ± 14/ ***	121 ± 11/	150 ± 12/ ***
24H DBP (mm Hg)	87 ± 11	78 ± 8	95 ± 8 ***	80 ± 8	96 ± 8 ***
Day SBP/ (mm Hg)	139 ± 20/	123 ± 12/	152 ± 15/ ***	124 ± 11/	155 ± 13/ ***
Day DBP (mm Hg)	92 ± 12	81 ± 7	101 ± 8 ***	83 ± 8	102 ± 8 ***
Night SBP/ (mm Hg)	130 ± 17/	119 ± 14/	140 ± 14 ***	119 ± 12/	143 ± 12 ***
Night DBP (mm Hg)	83 ± 12	75 ± 9	90 ± 9 ***	76 ± 9	91 ± 9 ***

SBP, systolic BP; DBP, diastolic BP; 24H, 24-hour; BMI, body mass index. *** p<0.0001 versus participants with mean day ABP < 140 or 90 mm Hg

TABLE 2: Differences in amino acid concentrations (µmol/L) in participants with normal and elevated ambulatory blood pressures (ABP). Bold shows data that is different from participants with a normal day BP.

	All subjects (n=75)	Mean Day ABP DBP<90mm Hg (n=33)	Mean Day ABP DBP≥90mm Hg (n=42)	Mean Day ABP SBP<140mm Hg (n=39)	Mean Day ABP SBP≥140mm Hg (n=36)
Alanine	177 ± 41	186 ± 42	169 ± 38	181 ± 42	172 ± 39
Arginine	57 ± 16	56 ± 13	58 ± 18	54 ± 15	**61 ± 17***
Asparagine	23 ± 22	23 ± 14	22 ± 27	21 ± 13	24 ± 29
Citrulline	14 ± 7	13 ± 4	15 ± 9	13 ± 4	17 ± 10
Histidine	40 ± 6	41 ± 6	40 ± 6	41 ± 6	40 ± 6
Isoleucine	36 ± 10	35 ± 9	37 ± 10	35 ± 9	37 ± 10
Leucine	69 ± 14	67 ± 11	70 ± 16	67 ± 12	70 ± 16
Lysine	77 ± 19	76 ± 18	78 ± 20	74 ± 19	81 ± 19
Methionine	11 ± 4	12 ± 4	11 ± 4	12 ± 4	11 ± 3
Ornithine	17 ± 15	15 ± 12	18 ± 16	17 ± 13	17 ± 16
Phenylalanine	35 ± 7	34 ± 6	35 ± 7	34 ± 7	35 ± 6
Proline	86 ± 94	71 ± 16	97 ± 125	69 ± 18	103 ± 133
Serine	9 ± 15	13 ± 20	**5 ± 6***	11 ± 18	6 ± 6
Taurine	29 ± 18	28 ± 18	30 ± 18	28 ± 18	31 ± 18
Threonine	59 ± 21	61 ± 20	57 ± 22	60 ± 22	57 ± 20
Tryptophan	24 ± 6	24 ± 6	24 ± 6	23 ± 6	25 ± 6
Tyrosine	22 ± 6	22 ± 6	22 ± 6	22 ± 6	22 ± 5
Valine	90 ± 19	87 ± 17	93 ± 20	88 ± 18	93 ± 20
3-methylhistidine	5 ± 4	5 ± 4	4 ± 3	5 ± 4	4 ± 3

SBP, systolic BP; DBP, diastolic BP. * =p<0.05 versus group with mean day ABP < 140 or 90 mm Hg

TABLE 3: Pearson correlation coefficients between fasting plasma arginine and lysine concentrations and ambulatory blood pressures. Bold indicates significant correlations.

	Arginine				Lysine
	All participants (n=75)	BMI<30kg/m2 (n=55)	Males (n=60)	Males BMI<30kg/m² (n=46)	Mean Day ABP BMI<30kg/m² (n=46)
Mean Ambulatory BP					
24H SBP/ (mm Hg)	0.16	0.26	0.23	**0.31***	**0.32***
24H DBP (mm Hg)	**0.26***	0.23	**0.32***	**0.37***	**0.31***
Day SBP/ (mm Hg)	0.16	**0.27***	0.20	0.26	0.25
Day DBP (mm Hg)	0.22	**0.33***	**0.27***	**0.30***	0.21
Night SBP/ (mm Hg)	0.16	**0.27***	**0.25**	**0.35***	**0.42****
Night DBP (mm Hg)	**0.28***	**0.36***	**0.36****	**0.45****	**0.46****

*SBP, systolic BP; DBP, diastolic BP; 24H, 24-hour.   * p<0.05, ** p<0.005*

No significant differences were noted in the characteristics of the participants with and without an increased day BP except for higher BP values noted in those with increased day BP values.

Plasma amino acid profiles in participants

Table 2 shows the amino acid profiles in all participants and the differences in amino acid profiles of participants with a normal versus increased day BP. A lower plasma serine concentration was noted in participants with elevated day diastolic BP values. Moreover, an increased plasma arginine concentration was noted in participants with an increased day systolic BP.

Relationship between plasma amino acid concentrations and ambulatory BP

Table 3 shows the Pearson's correlation coefficients between plasma arginine and lysine concentrations and ambulatory BP. Plasma arginine concentrations were positively correlated with ambulatory BP in the whole group and in secondary analyses in males and non-obese participants. Plasma concentrations of lysine, an amino acid using the same membrane transporter as arginine, also correlated with ambulatory BP in non-obese males. In all participants, two other amino acids showed statistically significant correlations with BP. In this regard, plasma alanine concentrations correlated negatively with mean day systolic BP (r=-0.23, p<0.05) and plasma serine concentrations correlated negatively with mean day diastolic BP (r=-0.25, p=0.046). Plasma serine, but not alanine concentrations, remained significantly and inversely correlated with both mean daytime systolic (r=-0.42, p=0.007) and diastolic (r=-0.43, p=0.006) BP in males with a BMI<30kg/m².

Factors associated with ambulatory BP in multivariate regression models

In a multivariate model with age gender, body mass index, arginine, alanine, proline and serine included in the model, only male gender (p<0.05) and plasma arginine concentrations (p<0.05) were independently associated with night diastolic BP. When including lysine in a regression model with arginine, lysine replaced arginine as being a significant predictor of mean night diastolic BP.

DISCUSSION

The main finding of the present study is that plasma concentrations of the NO precursor, L-arginine, are positively associated with night diastolic BP in a group of 75 participants of African descent in Africa and that this association remained after adjustments for potential confounders in addition to the plasma concentrations of other amino acids. This positive association between plasma arginine concentrations and night diastolic BP was similarly observed in secondary analysis in the predominant gender (male) in the study group and in participants whom were not obese.

The results of the present study conducted in a group of African descent in Africa, concur with two studies conducted in Caucasian groups with a case-control study design showing that plasma arginine concentrations are increased in hypertensive patients.[14,15] In this regard however, in one study,[14] plasma samples were obtained in 36 normotensive controls and 34 hypertensives whilst these patients were still on therapy. Therefore, whether the results of this prior study[14] represent an impact of treatment or the presence of hypertension is uncertain. In the other study, plasma arginine concentrations were shown to be increased in 36 never-treated hypertensives as compared to only 8 control participants.[15] It may be argued that these data reflect a false positive outcome as the study sample of control participants was very small. The results of the present study therefore add to these prior studies as the analyses performed was conducted using a continuous (regression analysis) together with a case-control approach, the study sample size of never-treated participants (n=75) was considerably larger than prior studies, and ambulatory BP was performed to improve the sensitivity of the analysis.

The positive association between plasma arginine concentrations and BP in the present study requires an explanation. In this regard it is unlikely to reflect cause and effect as extracellular arginine is required for NO production[5] and consequently vasodilatation[19,20] a change that will decrease and not increase BP. Moreover, supplementation with dietary arginine decreases BP in hypertension.[6] It is possible that the positive relationship between plasma arginine concentrations and BP may reflect a reduced capacity for the cellular uptake of arginine as previously suggested,[14,15] a change which would increase plasma arginine concentrations whilst at the same time reduce the capacity for NO synthesis.

We are unable to provide an explanation for either the lower plasma serine concentrations in participants with an elevated BP or the positive relationship between plasma lysine concentrations and ambulatory BP in males with a BMI <30 kg/m². However, studies conducted in a genetic animal model of hypertension[21] have similarly reported decreased serine concentrations in hypertension. Moreover, plasma lysine concentrations may be closely correlated with plasma arginine concentrations as arginine and lysine depend on the same membrane transporter. This relationship may explain the ability of plasma lysine to replace plasma arginine concentrations as independent predictors of BP in multivariate regression analysis.

The results of the present study need to be interpreted in the context of the study limitations. In this regard, the present study was conducted in a study sample with a high proportion of men (80%). Hence, the outcomes may reflect a gender-specific effect. Second, the present study was performed in a convenience sample and hence further studies are required in a larger study sample of randomly recruited participants. Third, the present study was a cross-sectional study and hence further studies are required to establish a temporal relationship between plasma arginine concentrations and ambulatory BP. Last, we did not measure membrane transport of arginine or NO production from cells in participants and hence we could not provide an explanation for the positive relationship between plasma arginine concentrations and BP.

In conclusion, a positive relationship between plasma arginine concentrations and ambulatory BP occurs in people of African origins. These data support the notion[14-16] that although increases in BP are unlikely to be attributed to a decreased availability of arginine, increases in BP may occur because of a deficiency in arginine uptake resulting in a reduced synthesis of NO.

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